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The Clinical and Molecular Spectrum of Androgen Insensitivity Syndromes

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Androgen insensitivity syndromes (AIS) are due to end-organ resistance to androgenic steroids in males leading to defective virilization of the external genitalia. The phenotype encompasses a wide array of genital ambiguity and may range from completely female to undervirilized but unequivocally male with infertility. This disorder is caused by mutations of the androgen receptor and is an X-linked recessive trait. We have studied 47 patients with AIS and have characterized the underlying molecular abnormality in the androgen receptor gene.

Twenty patients had complete AIS and twenty-seven had partial AIS. Of the latter, 11 were of predominantly female phenotypic appearance and gender was assigned accordingly, while 16 were raised as males. Within the group of complete AIS, two patients had gross deletions within the gene, one had a small deletion, and one had an insertion. In the other patients with complete AIS, as well as all individuals with partial AIS, single nucleotide substitutions within the coding region were detected, each leading to an amino acid alteration. Seven codons were involved in more than one mutation in different cases. In addition, in one patient with spinal and bulbar muscular atrophy, an elongation of a glutamine-repeat was characterized.

We conclude that mutations in the androgen receptor gene may be present throughout the whole coding region. However, our study provides evidence that several mutational hot spots exist. © 1996 Wiley-Liss, Inc.

KEY WORDS: androgen receptor, genetics, androgen insensitivity syndrome, male pseudohermaphroditism

INTRODUCTION

Androgen insensitivity syndromes (AIS) are a well known entity in the nosology of male pseudohermaphroditism. In 1817 Steglehner reported a female lacking Müllerian structures and with gonads resembling testes. In 1953, Morris used the term "testicular feminization" in the description of individuals with female phenotype and satisfactory breast development despite endocrine active testes. The phenotypic spectrum was expanded by the retrospective inclusion of reports of male patients with hypospadias, marked undervirilization, and gynecomastia after puberty [Reifenstein, 1947; Lubs et al., 1959]. All of these individuals had a normal 46,XY karyotype. Evidence that infertility in otherwise normal males may be due to AIS was presented by Aiman et al. [1979]. Keenan et al. [1974] characterized defective binding of natural and synthetic androgens in lysates of genital skin fibroblasts in patients with AIS. This has led to the concept that defects within the receptor transmitting the action of androgens should be the cause of the disorder. In 1988, several groups were able to clone and sequence the gene encoding the androgen receptor (AR) which had been mapped to Xq11-12 [Lubahn et al., 1988; Chang et al., 1988; Trapman et al., 1988]. The gene consists of eight exons coding for three major functional domains. A large aminoterminal end is followed by a highly conserved DNA-binding region and a ligand binding domain [Brinkmann et al., 1989]. Shortly afterwards, mutations within the coding region of this gene were detected in patients with AIS [review in Quigley et al., 1995]. These were found to occur mostly within the DNA- and hormone-binding region. In addition, a correlation of an expansion mutation within a glutamine-repeat sequence encoded by exon 1 to the development of an adult spinal and bulbar muscular atrophy (M. Kennedy) associated with clinical signs of mild androgen insensitivity was described by LaSpada et al. [1991].

Advances of molecular screening techniques for the detection of point mutations within known sequences amplified by the polymerase chain reaction (PCR) has facilitated the use of these methods for the primary diagnosis of androgen resistance by the characterization

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Dedicated to Jürgen W. Spranger on the occasion of his 65th birthday with admiration and best wishes.

of the underlying mutation in the androgen receptor gene [DeBellis et al., 1992; Hiort et al., 1993, 1994]. We have investigated patients presenting with the diverse phenotypes of androgen resistance for mutations in the androgen receptor gene and report 47 cases analyzed on the molecular level.

MATERIAL AND METHODS

Patients

Patients were referred by physicians cooperating in the German Collaborative Intersex Study. Their age at the time of DNA analysis was between only a few days for newborn infants with ambiguous genitalia up to 56 years for a male with clinical signs of spinal and bulbar muscular atrophy. The phenotype varied widely. Twenty patients presented with a completely female phenotype; six of these presented postpubertally with primary amenorrhea, while the others were referred before puberty.

In the patients with signs of virilization, diagnostic work-up had been initiated at an early age. However, in most, a diagnosis was not made and gender assignment was based on phenotypic appearance and consensus decisions of pediatrician and surgeon. At the time of availability of molecular genetic investigation, DNA analysis was initiated. Eleven patients had been assigned a female gender, because of only slight signs of virilization with one or more of the following: clitoral enlargement, fusion of the labia minora, and rugation of the labia majora. Sixteen patients were raised as males. They all had genital malformation consisting of micropenis, hypospadias, and development of gynecomastia during puberty. Between the groups matched to a female gender and those assigned to a male gender there was a considerable overlap of patients with frankly ambiguous genitalia.

In 23 patients of different phenotype, an in vivo androgen insensitivity test based on the diminished down regulation of the sex hormone-binding globulin (SHBG) after the oral administration of the anabolic steroid stanozolol had been initiated and had demonstrated androgen resistance (Sinnecker and Köhles, 1989, unpublished observation).

Figure 1a,b,c shows the appearance of patients with the different phenotypes of AIS.

DNA Analysis

Analysis for point mutations within the coding region of the androgen receptor gene was performed as previously described employing non-isotopic single strand conformation analysis (SSCA) [Hiort et al., 1993, 1994]. Samples showing aberrant migration at SSCA were reamplified from genomic DNA and directly sequenced in two separate reactions as described earlier [Hiort et al., 1993]. Southern blot analysis for larger structural defects was adapted from Quigley et al. [1992]. For analysis of the variable glutamine (CAG) repeat region within exon 1, amplified fragments were electrophoresed on a non-denaturing polyacrylamide gel. For sizing of the CAG-repeat, the gels were evaluated using the computerized analysis system ImageMaster (Pharmacia, Freiburg, Germany).

RESULTS

In all patients, a distinct defect within the AR gene was analyzed. In only two cases, major gene defects were detected. In one patient who presented with a completely female phenotype and absent secondary hair ("hairless women"), a complete deletion of the AR gene was found, while in another patient with complete AIS a deletion of exon 3 was characterized. Three mutations lead to a premature stop codon and were associated with complete androgen insensitivity. The premature termination codons were induced either by frameshift mutations due to deletion or insertion of nucleotides or due to a single base substitution in one case. In all other patients, point mutations leading to amino acid substitutions were detected. While the premature termination codons were localized within exon 1, all amino acid substitutions were situated in one of the exons 2 to 8, encoding the DNA- and hormone-binding regions of the receptor (Fig. 2).

In a male with spinal and bulbar muscular atrophy an expansion to 40 glutamine repeats within exon 1 was characterized.

While most of the point mutations were spread throughout the gene, seven codons were subject to different mutations (Fig. 2). The two mutations affecting codon 582, Phe-Tyr and Phe-Ser [nomenclature of amino acid numbering according to Lubahn et al., 1989] were both associated with a predominantly female phenotype, although partial androgen insensitivity was found in the girl with the latter mutation [Hiort et al., 1994]. Also a complete AIS was found in the patients with different mutations in codon 774 (Arg-Cys, Arg-His). In contrast, the mutations affecting codons 615, 754, 855, 866, and 870 were related to either complete or partial AIS with either female or male gender assignment, depending on the induced variation. In addition, three mutations, 774 Arg-Cys, 840 Arg-His, and 870 Ala-Val, were each found in two unrelated patients.

DISCUSSION

Androgen insensitivity syndromes comprise a variable spectrum of virilization disorders in karyotypic males. The analysis of point mutations in the AR gene has lead to an enormous advancement of our understanding of the clinical appearance of these patients. To this date about 200 point mutations have been described within the AR gene and a regular updated data base is compiled by Dr. Bruce Gottlieb, Lady Davis Institute for Medical Research in Montreal, Canada.

In our study we have only found two major gene defects and they were both related to the complete form of AIS. Also, nonsense mutations leading to a presumably truncated AR protein due to disrupted translation are usually associated with a completely female phenotype. However, in most patients (15/20) with complete AIS, point mutations with subsequent amino acid substitutions are found. These mutations all constitute nonconservative amino acid exchanges and it must be presumed that a major dysfunction of the AR results.

In patients with partial AIS, only missense mutations were detected. These were limited to the exons 2

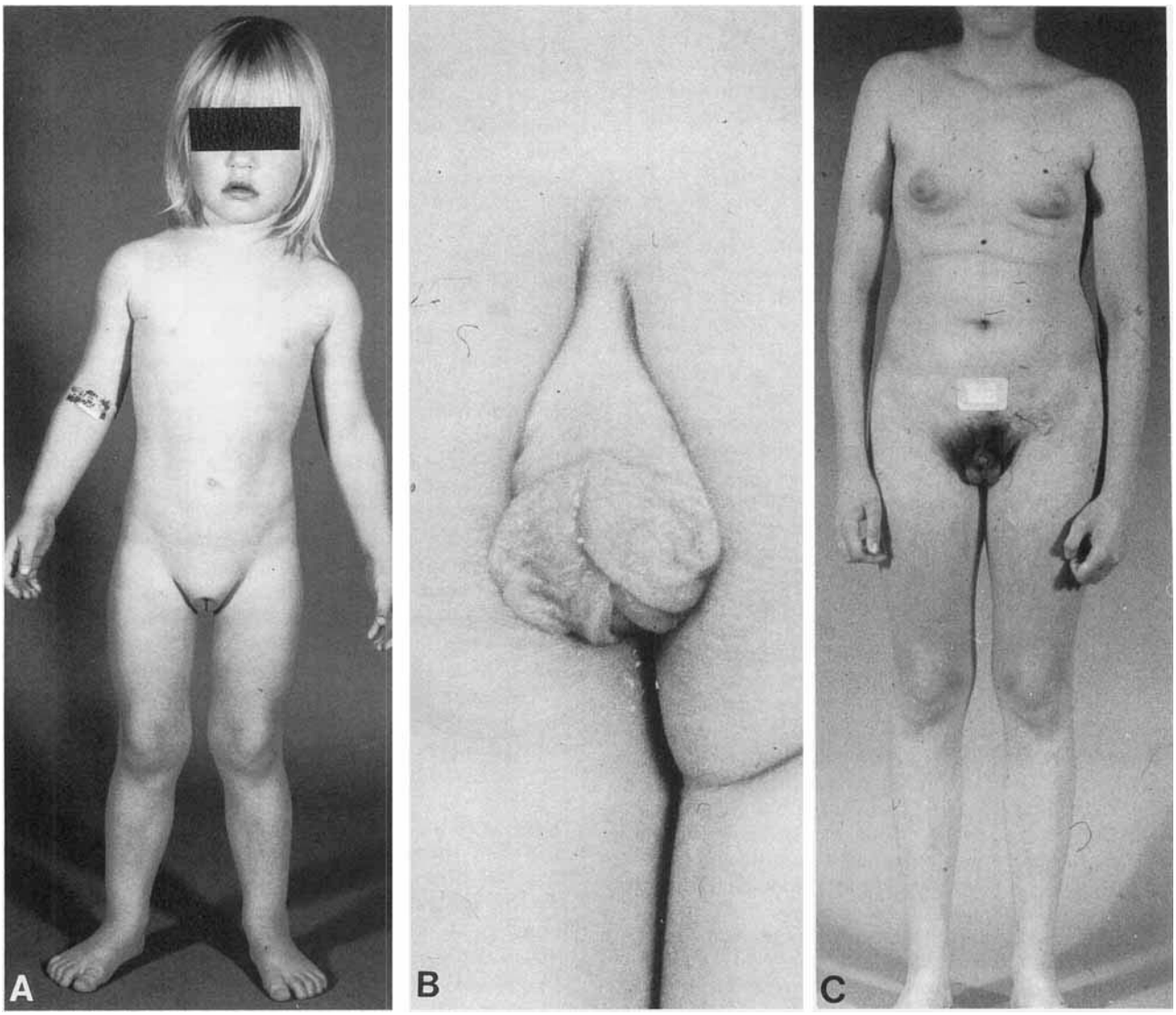

Fig. 1. Phenotypic manifestations of androgen insensitivity syndromes. **A** shows a patient with complete AIS (from Sinnecker GHG (1993): Störungen der Keimdrüsen und der sexuellen entwicklung. In Kruse K (ed): "Pädiatrische Endokrinologie." Stuttgart: Enke Verlag, p 150). **B** demonstrates ambiguous genitalia in a patient with partial AIS. **C** is an example of a patient with partial AIS and predominantly male phenotype (Reifenstein Syndrome).

to 8, encoding for the DNA- and hormone-binding regions of the receptor. This finding emphasizes that point mutations within exon 1 associated with partial AIS are rare. However, an unusual mutation of the AR gene was found in a male with the neurological disorder of spinal and bulbar muscular atrophy. The expansion of the variable glutamine region to 40 repeats is outside of the normal variability and has been associated with the onset of neurological symptoms in adulthood [LaSpada et al., 1991]. This disorder is an example for the diverse molecular mechanisms controlled by the AR.

Seven amino acid positions were subject to more than one mutation. Interestingly, in five codons different mutations lead to strikingly different clinical phenotypes. While the mutations 615 Arg-Pro, 754 Phe-Leu, 855 Arg-His, 866 Val-Leu, and 870 Ala-Val were associated with a predominantly male phenotype (as in Fig. 1c), the mutations 615 Arg-His, 754 Phe-Val, 855 Arg-Cys, 866 Val-Met, and 870 Ala-Gly were either associated with a predominantly female phenotype or even complete AIS. At least for mutations affecting codons 866 and 870 it can be suspected that conservative amino acid substitutions lead to a less severe impairment of AR function than non-conservative exchanges.

The mutations 774 Arg-Cys, 840 Arg-His, and 870 Ala-Val were found twice in unrelated subjects. These individuals had similar phenotypic appearance, thus allowing for a limited genotype-phenotype correlation. Altogether, the single nucleotide mutations characterized

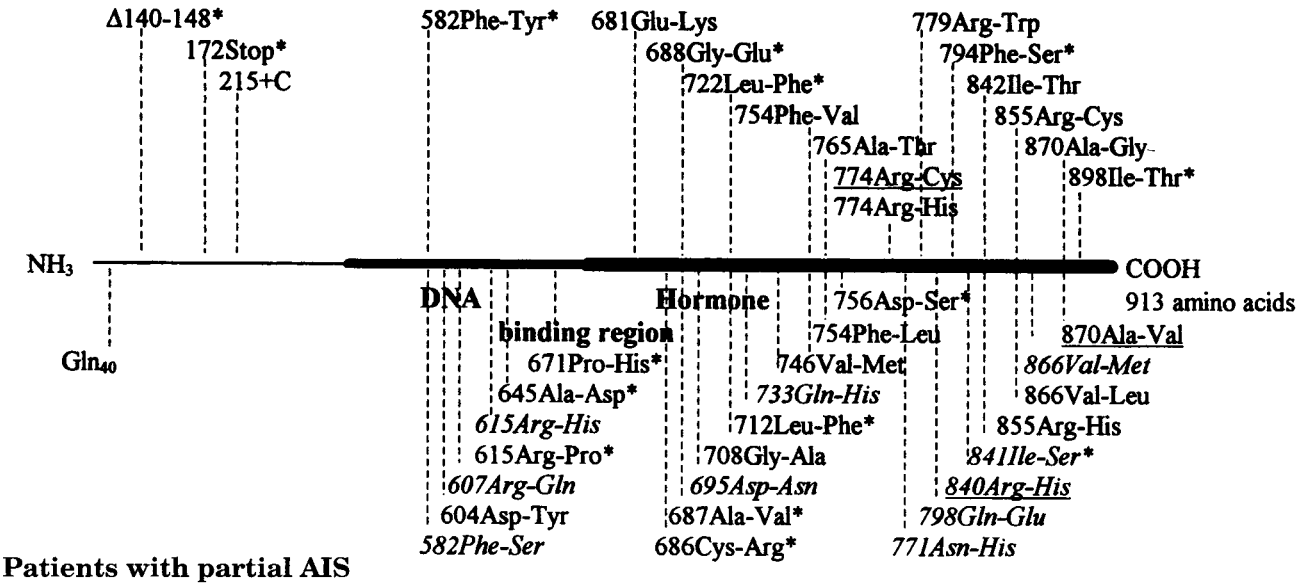

Fig. 2. Graphic design of the AR with N-terminal end, DNA- and hormone-binding regions (not to scale). The nonsense and missense mutations found in patients with complete and partial AIS and their respective locations in the different functional domains of the receptor are shown. Within the group of patients with partial AIS, the mutations associated with female gender assignment are printed in italic. The mutations that are first described in this report are marked by an asterisk. Those mutations that were characterized independently in two unrelated individuals are underlined.

in 43 unrelated patients were limited to 33 codons, thus providing evidence that hot spots within this gene exist.

Our study shows that regular molecular genetic analysis of the AR gene is an effective and sensitive diagnostic procedure. It can be performed irrespective of age and together with clinical endocrine function tests provides the basis for therapeutic decisions regarding gender assignment and pubertal development.

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